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Investigation of Fuel Production Using Metalloporphyrin-Based Complexes as Catalysts and Electron-Transfer Intermediates

ANNUAL REPORT

(April 1987 - December 1988)

INVESTIGATION OF FUEL PRODUCTION USING METALLOPORPHYRIN-BASED COMPLEXES AS CATALYSTS AND ELECTRON-TRANSFER INTERMEDIATES

ANNUAL REPORT

(April 1987 - December 1988)

Prepared by

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Sandia National Laboratories

Albuquerque, NM 87185

For

GAS RESEARCH INSTITUTE

Contract No. 5082-260-0767

GRI Project Manager

Kevin Krist

Basic Research Division

January 1989

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Metalloporphyrins have appropriate properties for photosensitizing and catalyzing solar energy storage reactions. Fundamental spectroscopic studies of metalloporphyrins and related enzymes that carry out C_1 chemistry can identify the factors controlling reactivity of the metal complexes. Research has concentrated on mimicking biological methanogenesis through investigation of the enzyme methylreductase, which carries out the final step in the reduction of CO_2 to methane. Transient and difference Raman spectroscopies were used to investigate the structural features of methylreductase, its nickel-hydrocorphin Cofactor F_{430}, and hydrocorphin and porphyrin analogs of the active nickel complex. In particular, axial ligation at the nickel site was evaluated under a variety of conditions with the goal of elucidating the mechanism of methane synthesis. Studies of the tin-and antimony-porphyrin photoredox cycles were also carried out as possible solar-driven sources of reductant for biomimetic methane generation.

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Contractor	Sandia National Laboratories
Principal Investigator	J. A. Shelnutt
Report Period	May 1987 - December 1988 Annual Report
Objective	To develop metalloporphyrins as sensitizers and catalysts in biomimetic inorganic fuel generating systems.
Technical Perspectives	Metalloporphyrins have unique properties for catalyzing and photosensitizing solar energy storage reactions. The fundamental studies of these molecules is vital to understanding biological energy-storing reactions and mimicking these reactions for gaseous fuel production. The lack of information about the relationships between catalytic reactivity and the molecule's structure and micro-environment hampers efforts to design energy storage chemistries based on these metal complexes. Fundamental spectroscopic studies of metalloporphyrins in fuel-producing enviroments and of the intact enzymes that carry out C_1 chemistries are being carried out to identify the factors controlling reactivity. The research has emphasized biomimetic approaches to CO_2 conversion to CH_4 and is related to GRI efforts on methane activation. The primary focus has been an investigation of the terminal enzyme of methanogenesis, methylreductase, and the nickel hydrocorphin that is the catalytic metal complex at the active site of the methylreductase. Methylreductase reduces methyl groups derived from CO_2 to methane. The reductant in methanogenic bacteria is provided ultimately by H_2, but in a biomimetic process might be provided by a solar energy driven reaction. The methylreductase reaction is also intimately coupled to the activation of CO_2 in methanogenesis. The reverse reaction of

methylreductase is of special interest for CH_4 activation.

Results

Active Site Complexes and Intact Enzymes. Raman difference spectroscopy, transient Raman spectroscopy, and X-ray absorption techniques have been applied to determine the micro-environment of the nickel-hydrocorphin cofactor, called F_{430}, at the active site of methylreductase. Room temperature and frozen solutions of F_{430} and its stereo-isomers were investigated, and under all conditions the spectrum of the cofactor was dissimilar from the spectrum of the intact enzyme. Raman studies of the cofactor in aqueous solution showed that the cofactor is a mixture of 4-coordinate (no axial ligands) and 6-coordinate (two H_2O ligands) forms at room temperature, but converts fully to the 4-coordinate form at 60° C where the enzyme is catalytically active. Further, the spectra of the cofactor with a large variety of axial ligands show little similarity to the, as of now, unique spectrum of methylreductase. However, preliminary studies of ligand binding to methylreductase have shown that strong field ligands can convert the unique spectrum of methylreductase to a spectrum similar to the solution F_{430} complexes with strong field ligands. The X-ray absorption spectra suggest at least one strong field ligand provided by the protein moiety. Thus, the results suggest that F_{430} has one strong and one weak field ligand in its two axial ligation positions. We have recently discovered that the 4-coordinate cofactor exhibits conformational heterogeneity--probably a range of conformers with differing degrees of out-of-plane ruffling of the hydrocorphin macrocycle.

Synthetic Cofactor F_{430} Analogs. A variety of Ni-porphyrin and hydrocorphin analogs of the methylreductase cofactor have been investigated using Raman techniques. A previous study had shown that the high frequency modes of a nickel-hydrocorphin analog of F_{430} were indicative of the state of axial ligation. This behavior was similar to that of the porphyrins. In new Raman

studies of the cofactor and its analog, we discovered that multiple 4-coordinate forms coexist in solution. These forms were suspected to show differing degrees of out-of-plane distortions of the macrocycle on the basis of the crystal structures of model 4-coordinate Ni complexes. Since even nickel porphyrins, which are much more rigid than the hydrocorphins, show ruffled macrocycles in one crystalline form, we therefore decided to re-investigate the solution behavior of nickel octaethylporphyrin in noncoordinating solvents. We found that even the nickel porphyrins exist as a range of conformations from planar to highly ruffled. Macrocycle ruffling is thought to play a role in the axial ligation processes involved in the catalytic mechanism of methylreductase. We also investigated the excited d-d state of the F_{430} analogs that is active in the addition and ejection of axial ligands.

CO_2 to CH_4 Process Development. Finally, since the ultimate goal of the work is to develop a process for conversion of CO_2 to methane, we have continued our investigation of the novel solar-driven redox chemistry based on the tin and antimony porphyrins. With this reductive photocycle it may be possible to drive a reaction mimicking biological methane synthesis. As a result of recent work using time-resolved spectroscopic methods the photocycle is now fully understood. In work performed for DOE the photocycle has been used to drive biomimetic room-temperature conversion of alkanes to alcohols. With a more thorough understanding of the enzyme pathways involved in methanogenesis, solar-driven methane synthesis may also be possible.

Technical Approach

Nickel porphyrins and nickel hydrocorphins incorporated into various micro-environments are examined by Raman and X-ray spectroscopic techniques. These probes are being used to determine molecular structure and to identify photogenerated products and reaction intermediates. These studies form the basis for understanding the spectra of solutions of methylreductase and its cofactor. These

experimental measurements are complemented by molecular modeling calculations for the model complexes and F_{430}.

Project Implications

This research is studying porphyrin-based molecules that could eventually absorb light and catalyze methane formation from water and carbon dioxide. The research is attempting to mimic the biological enzyme methyl-reductase, which carries out the final step in the reduction of CO_2 to methane. During the previous year, the research determined the axial ligation at the nickel-porphyrin active site in the enzyme and enzyme analogs in order to determine the mechanism of methane synthesis, and carried out studies of tin and antimony porphyrin photoredox cycles to generate methane from sunlight. GRI is discontinuing its inorganic SNG research in 1989. This contract will continue until the end of 1989, after which GRI will review the possibility of its redirection to research on conversion of methane to higher valued chemicals.

GRI Project Manager
Kevin Krist
Manager, Inorganic Chemistry Basic Research

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INTRODUCTION

Metalloporphyrins have shown considerable potential as photosensitizers and catalysts in a number of solar energy conversion chemistries. To exploit their good solar absorption attributes, photoredox properties, and catalytic characteristics, the detailed effects of modifications of their energy-level and molecular structure on electron-transfer and catalytic rates need to be understood.

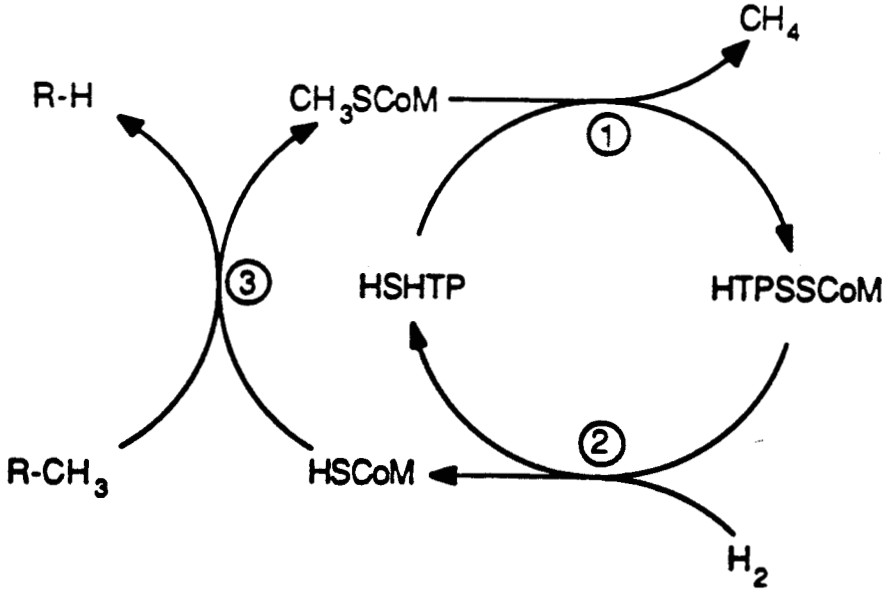
We are investigating the catalytic and photochemical properties of a variety of metal-tetrapyrrole complexes using spectroscopic means. These methods include Raman difference spectroscopy and time-resolved Raman spectroscopy (performed at Sandia by J. A. Shelnutt and the University of New Mexico by M. R. Ondrias), time-resolved absorption and fluorescence methods (performed at Ecole Polytechnique Federale de Lausanne by K. Kalyanasundarum and M. Grätzel) and EXAFS and XAS spectroscopic techniques (performed at Stanford by R. A. Scott and A. K. Shiemke of the University of Georgia). The goal of the spectroscopic work is to develop a model of the variation of fuel-production rates as a function of molecular structure of the metalloporphyrin photosensitizers and catalysts. These spectroscopic studies are complemented by molecular energy optimization and dynamics calculations as well as molecular orbital calculations.

The goal is to use the evolving structure-reactivity relationship to guide the design of new photosensitizers and

catalysts. In this way we will be able to tailor the properties of the metalloporphyrins to give optimum performance in solar-driven fuel producing systems.

One of the nice features of the metalloporphyrins is that nature has used these metal complexes to perform many energy transducing chemistries in biological systems. We are studying these reactions, which nature has optimized for her specific purposes in the organism, to learn how to tailor the metalloporphyrins for commercial applications. Specifically, we are interested in mimicking the enzymes that carry out C_1 chemistry for the purpose of generating and converting natural gas.

We have so far focused our studies on biological methanogenesis, and, in particular, the methylreductase enzyme, which carries out the 2-electron reduction of a methyl group originating from CO_2 to methane. Scheme **1** shows schematically the reaction catalysed by methylreductase (reaction **1**). The reducing



Scheme 1

equivalents for the reaction are provided by HSHTP (N-7-mercaptoheptanoyl-O-phospho-L-threonine), which is derived from enzymatic H_2 splitting (reaction **2**). Reaction **2** also regenerates the mercapto form of Coenzyme M (HSCoM) from the disulfide product of the methylreductase reaction. HSCoM, in another enzymatic reaction **3**, picks up the methyl group that is reduced to methane in a methyl-transfer reaction **3**.

The specific role of the nickel(II) tetrapyrrole F_{430} in the reductive cleavage of CH_3SCoM is unknown, but it is thought to be the site of substrate reduction. Possible roles for F_{430} in this reaction include substrate binding (methyl-SCoM and/or HSHTP), electron transfer from HSHTP to methyl-SCoM, or direct methyl-group transfer. The existence of Ni(I) forms of the enzyme indicate the reductive role of F_{430}.[1] It is speculated that the different forms of the enzyme observed may differ with respect to the nature of the axial ligands. Thus, the study of axial coordination chemistry of F_{430} is crucial to the understanding of the novel C_1 chemistry of the methylreductase enzyme. Consequently, much of our research for the current year has focused on the axial ligation chemistry of F_{430}, methylreductase, and model nickel hydrocorphins and porphyrins.

Ruffling of the tetrapyrrole macrocycle has an effect on axial ligation, and the out-of-plane conformation of the macrocycle offers a plausible mechanism by which the protein component of methylreductase can control F_{430} reactivity. Consequently, considerable effort has gone into developing spectroscopic means

of detecting these ruffled forms in F_{430} and its analogs. Also, a number of molecular modeling studies have been carried out and have helped to improve our understanding of ruffling phenomena.

Finally, in preparation for coupling methane synthesis reactions to solar-driven redox chemistry, we have further investigated the tin and antimony porphyrins as photosensitizers for production of the required reducing equivalents. This investigation has verified the reductive nature of the photocycle and determined many of the rates and lifetimes of the intermediate species involved in the photocycle.

OVERVIEW OF CURRENT YEAR RESEARCH

Studies of Cofactor F_{430} and Methylreductase. During the current year we reported (Shiemke, A. K.; Scott, R. A.; Shelnutt, J. A., J. Am. Chem. Soc. **1988**, 110, 1645) the first resonance Raman spectroscopic study of methylreductase and pure F_{430} and its isomers. The work centered on the axial ligation properties of the cofactor. Eleven potential ligands were investigated. The thermally isomerized 12,13-diepimer of F_{430} gave a 4-coordinate species in aqueous solution, whereas, native F_{430} gave a mixture of 4- and 6-coordinate forms. More recent Raman and X-ray work (Shiemke, A. K.; Shelnutt, J. A.; Scott, R. A., submitted to J. Biol. Chem., see Abstract, Appendix 1) has identified the 6-coordinate form as the bis-aquo complex (*vide infra*).

Comparison of the Raman spectra of methylreductase and various axial ligand complexes of F_{430} and its diepimer showed that none of the solution 6-coordinate complexes gave a spectrum similar to methylreductase. The ligands included sulfur, nitrogen, and oxygen ligation sites. The axial ligation properties of F_{430} and its diepimer are discussed in detail elsewhere (Shiemke, A. K.; Kaplan, W. A.; Hamilton, C. L.; Shelnutt, J. A.; Scott, R. A., submitted to J. Biol. Chem., see Abstract, Appendix 2).

Although the nature of the axial ligands in the enzyme is unknown at present, one can say that the site of F_{430} binding in the protein is unique in some fashion based on the large differences

in the methylreductase spectrum and the spectra of the F_{430} complexes. In addition, the site appears to provide a homogeneous micro-environment for the cofactor based on the narrowing of the Raman lines of methylreductase relative to the solution complexes.

Low temperature (77° K) Raman studies of methylreductase and the cofactor (Shelnutt, J. A.; Shiemke, A. K.; Scott, R. A., Reprints, Fuel Chem. Div., Methane Activation, ACS, **1987**, 32, 272-279.) have demonstrated the temperature dependence of axial ligation. Again, although the axial ligand(s) of methylreductase have not been identified the Raman data suggest that at least one of the ligands is a strong field ligand. The diepimer shows evidence of ruffling in that multiple forms are observed in the low temperature Raman spectra (*vide infra*). Native F_{430}, on the other hand, displays higher affinity for ligands at low temperature going completely to the bis-aquo form at 77° K; however, no new conformers were detected. The Raman spectrum of methylreductase exhibits some temperature dependence with what appears to be a new form growing in at low temperature. This is an important result, since EXAFS, XAS, and EPR measurements are typically carried out at low temperatures with the assumption that no changes occur as a result.

In aqueous solutions of F_{430}, changes in axial ligation occur over a narrow temperature range (0°-60° C) as described in the J. Biol. Chem. manuscript (Appendix 1). Resonance Raman spectra obtained at room temperature contain features characteristic of

both 4- and 6-coordinate species. At about 0° C the bis-aquo complex predominates; at 60° C the 4-coordinate form is formed. Similar behavior is observed in other weakly coordinating solvents such as methanol and ethanol. The 4-coordinate form is predominant even at room temperature in solvents with strong electron withdrawing substituents such as 2,2,2-trifluoroethanol and 2-mercaptoethanol.

The strong temperature dependence of H_2O binding affinity may also be physiologically relevant, since the methylreductase enzyme is isolated from thermophilic bacteria with an optimum growth temperature of 65° C. Such a temperature may be required to facilitate dissociation of endogenous nickel axial ligands so that substrate (methyl-SCoM or HSHTP) binding can occur. We are currently investigating the spectroscopic properties of enzyme-bound F_{430} to determine if structural changes accompany the thermal activation of methylreductase.

Studies of Synthetic Cofactor F_{430} Analogs. As mentioned above, low temperature Raman spectra of the diepimer of F_{430} show evidence of multiple conformers. Multiple conformers were also observed for the nickel-hydrocorphin analog of F_{430} and described in detail elsewhere (Shelnutt, J. A., submitted to J. Phys. Chem., see Abstract, Appendix 3). Our results indicate that the conformation of the macrocycle has a profound effect on the coordination chemistry of the nickel ion in F_{430}. The stereochemical configuration of the substituents on pyrrole ring C (See Figure 1)

in the native cofactor induces a macrocycle conformational preference that enhances the affinity of the nickel for axial ligands. It is thought that the macrocycle is constrained to a more planar geometry than for the configuration of the substituents on ring C of the diepimer. In the resulting larger core, the nickel ion is less coordinatively saturated by the in plane ligands, and, therefore, axial ligation is favored. Since it is only the native isomer of F_{430} that is incorporated into methylreductase to generate the active enzyme it can be postulated that this macrocycle-based stereochemical control mechanism may play an important physiological role.

In order to obtain a secure basis for the determination of the conformation of the F_{430} macrocycle, we have re-investigated the structure of nickel(II) octaethylporphyrin (NiOEP). NiOEP plays a central role in studies of the molecular properties of tetrapyrroles and tetrapyrrole-containing enzymes. Its importance stems from its use in isotopic substitution work for vibrational analysis of porphyrins and other tetrapyrroles, molecular orbital calculations, X-ray crystallographic structural studies, and many structural studies using a wide variety of spectroscopic techniques. These fundamental studies have had a significant influence on the development of our understanding of metalloporphyrin structure and bonding.

NiOEP is known to crystallize in two dramatically different structures, one of which is non-planar. In collaboration with Bob Scheidt at the University of Notre Dame, we have elucidated the

structure of these two forms and also a new third planar form using X-ray crystallographic and single-crystal Raman measurements (Brennan, T. D.; Scheidt, W. R.; Shelnutt, J. A., J. Am. Chem. Soc. **1988**, 110, 3919-3924). The Raman marker lines clearly distinguish the three phases. Large differences between the two planar phases and the ruffled form are observed. Smaller differences in the Raman spectra of the two planar forms were attributed to π-π stacking interactions between the macrocycles in one of the planar forms but not in the other.

From the investigation of NiOEP crystals of known structure, we obtained a basis for identifying ruffled and planar structures under other conditions. Further, since the diepimer of F_{430} and the model nickel-hydrocorphin both showed evidence of multiple forms in solution, we decided to re-investigate carefully the resonance Raman spectra of NiOEP in solution to determine whether other conformations coexist with the planar form (Alden, R. G.; Crawford, B. A.; Doolen, R.; Ondrias, M. R.; Shelnutt, J. A., J. Am. Chem. Soc. **1988**, 111, in press.). Indeed, NiOEP in noncoordinating solvents is found to be a mixture of planar and nonplanar, ruffled species at room temperature and at 77° K. The nonplanar conformation is ascertained to be the ruffled structure by the similarity of its spectrum to that of the crystalline ruffled phase. At 77° K, the S_4-symmetry ruffled form is more prominent than at room temperature. Also, the ruffled form is most prominent for laser excitation to the red of the Soret absorption maximum, suggesting that the ruffled form has a red-shifted Soret band.

Iterative extended Hückel molecular orbital calculation also suggest a red shift of the nickel-porphyrin absorption bands. Actually, a distribution of conformations is likely, given the large width and Gaussian nature of the line shapes required to fit the observed Raman data. Finally, the existence of both planar and ruffled species in solution explains some of the odd spectroscopic behavior of nickel porphyrins that is associated with aggregation and π-π complex formation. These phenomena are being re-investigated in light of these new results.

Because the spectroscopic manifestation of ruffling of NiOEP is very similar to what is observed for the F_{430} diepimer and its analogs it is likely that more or less ruffled and planar conformations account for the multiple forms. The implications of this conformational heterogeniety for methylreductase remains to be determined.

To develop a better understanding of ruffling phenomena, we have used molecular mechanics calculations to model the dynamical behavior of nickel tetrapyrroles. For NiOEP, a low frequency ruffling motion was detected. The period of the ruffling ranged from about 0.5 to 1.0 ps. Thus, such a normal mode would be less than the thermal energy, and, solvent trapping forces may lock the macrocycle into a distribution of conformations determined by the ruffling motion. This would account for the observed distribution of structures.

Figure 2 shows a ruffled structure that was calculated for the nickel-hydrocorphin F_{430} analog. The conjugated bonds and pattern

of peripheral substitution is identical to F_{430}. The structure is the result of a dynamics and energy-minimization calculation using BIOGRAF software. Nickel-nitrogen bond parameters were obtained by examination of related 4-coordinate nickel-corphinate crystal structures. We find that the *cis*-configuration of the 4,19-hydro and 6-cyano substituents, which are oriented quasi-axially, permits only one orientation of the saddle of the ruffled structure. The orientation of the saddle is determined by the 4,6-substituents because tetrahedral bonding at these positions insures that these positions will lie above the macrocycle plane. Molecular modeling for the isolated molecule thus indicates a single ruffled structure at low temperature. Further molecular modeling studies are underway to characterize more completely the structure of the various forms identified by Raman spectroscopy.

Another way to investigate the relationship between structure and axial ligation is by transient Raman studies of the ligand binding excited d-d state of nickel porphyrins and hydrocorphin. A detail investigation of the d-d state was carried out on nickel porphyrins in a variety of noncoordinating solvents (Findsen, E. W.; Shelnutt, J. A.; Ondrias, M. R., J. Phys. Chem. **1988**, 92, 307.). The excited d-d state, which becomes the ground state when ligands are added, is formed in all of the solvents investigated. However, the solvent does cause some shifts in the Raman spectrum of the nickel porphyrins in the excited state.

In contrast, the nickel-hydrocorphin model of F_{430} shows radically different photodynamic behavior from the nickel porphyrins (Crawford, B. A.; Findsen, E. W.; Ondrias, M. R.; Shelnutt, J. A., Inorg. Chem. **1988**, 27, 1842.). Under experimental conditions where nickel porphyrins exhibited significant formation of the excited state, the F_{430} analog produced no observable phototransient species. Also, the reverse d-d transition for strong field 6-coordinate complexes, which normally would result in the loss of ligands, causes no photolysis. Strong field ligand photolysis does occur for 6-coordinate nickel porphyrins. Weak field ligands are photoejected for both porphyrins and the hydrocorphin. Therefore, while the photodynamic behavior is similar for weak field ligands, the nickel hydrocorphin differs significantly from the analogous porphyrins in other aspects. Differences in the dynamic motion resulting from greater macrocycle flexibility and electronic differences resulting from macrocycle reduction, may contribute to their unique photodynamic properties. This technique may provide a valuable new probe of the methylreductase active site.

<u>CO_2 to CH_4 Conversion Process Development.</u> Conversion of carbon dioxide to methane is an uphill 8-electron reaction (ΔG_0 = -131 kJ/mol of CH_4) and thus requires a source of energy. Although we are not yet close to a process mimicking methanogenesis we must come to grips with the problem of a source of energy to drive the series of reactions. For the methanogens the source of energy is

H_2. Solar-driven photoredox cycles are one possible replacement for hydrogen as an energy source.

Photoredox reactions involving metalloporphyrins have been investigated for some years for the purpose of splitting water for the production of H_2. Usually, a relay molecule such as methylviologen (MV^{2+}) is the primary electron acceptor. A tertiary amine can serve as a sacrificial electron donor. A long range goal is to design a system in which a photoredox is cycle coupled to biomimetic methane synthesis.

A reductive cycle in which the porphyrin sensitizer is reduced to the porphyrin radical π-anion is attractive for this purpose, because the relay molecule may not be required. That is, in a reductive cycle the porphyrin anion itself may provide the reducing equivalents required for CO_2 reduction. Consequently, we have studied further the photoredox reactions sensitized by the tin and antimony porphyrins (Kalyanasundaram, K.; Shelnutt, J. A.; Grätzel, M., Inorg. Chem. **1988**, 27, 2820-2825.). These efficient photosensitizers were discovered earlier and patented in our GRI funded work.

We have verified in great detail that these photosensitizers operate *via* an efficient reductive quenching mechanism, yielding stable porphyrin anions in aqueous solution. The absorption spectra of intermediates, such as the excited triplet state and the anion, were identified and used to determine the lifetimes of the transient species. Also, quenching of the triplet excited state

by molecular oxygen was discovered. Subsequently, quenching by O_2 has been found to result in efficient production of singlet O_2.

In work for DOE, we have recently succeeded in coupling the photocycle to biomimetic alkane oxidation. Cytochrome P_{450} is an enzyme that carries out the hydroxylation of alkanes under mild conditions. However, the iron(III)-porphyrin resting state of the enzyme must be reduced to Fe(II) so that O_2 can bind. The O_2 complex must then be further reduced to obtain ultimately the reactive oxo-Fe(IV) intermediate. Reduced methylviologen, produced in the photocycle, can provide the reducing agent needed for the Fe-porphyrin catalyzed reaction. For this purpose it was necessary to replace water by an organic solvent. We have recently demonstrated hexane oxidation to hexanol and hexanone in such a photochemically driven analog of the cytochrome P_{450} reaction (using both Fe- and Mn-porphyrin catalysts).

FUTURE WORK

Besides the ongoing work mentioned above, work is progressing in two other experimental areas. First, work is progressing in the area of picosecond Raman studies of nickel-porphyrin excited states. A detailed study of Ni protoporphyrin IX dimethylester on the faster time scale has shown the existence of a precursor state to the d-d excited ($^3B_{1g}$) state. We are currently trying to identify and characterize the short-lived transient state. Second, now that we can reliably detect and identify the ruffled forms of nickel porphyrins, we are evaluating the effect of ruffling on such phenomena as π-π aggregation and complex-formation processes. It is expected that out-of-plane ruffling will disrupt aggregation and complex formation that involves stacking of the macrocycle with itself or with other extended aromatic rings.

Also, the Raman difference spectrometer at Sandia has recently been converted to work as a nanosecond-resolution single-channel transient Raman spectrometer, and will soon be converted to the first transient Raman *difference* spectrometer. The new instrument will be useful for detecting low concentrations of transient reaction intermediates and for making accurate comparisons of excited state and transient intermediate species.

Finally, molecular modeling work is continuing with the goal of improving force field parameters for nickel tetrapyrroles. Also, we have just received Michael Zerner's ZINDO molecular orbital program. The program should provide a great improvement

in accuracy over our current iterative extended Hückel self consistent field calculations.

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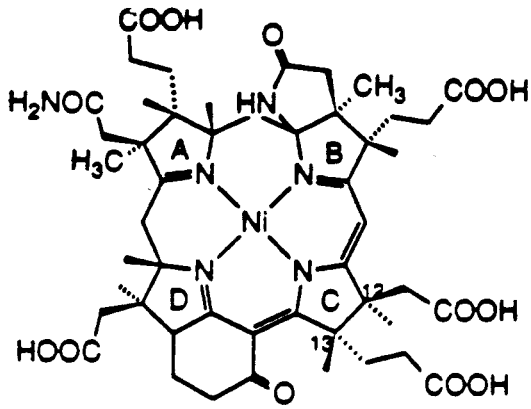
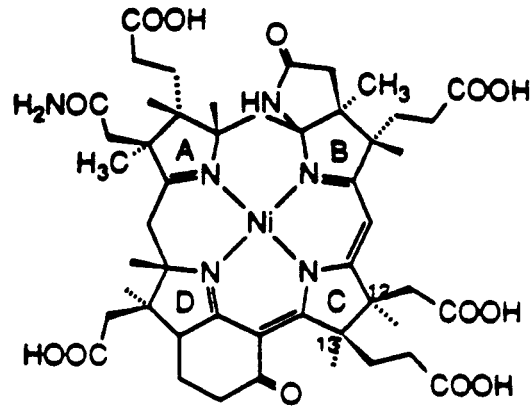
FIGURE CAPTIONS

1. Non-crystallographically determined structures of F_{430}, its configurational isomers, and the ring-oxidized derivative F_{560}.

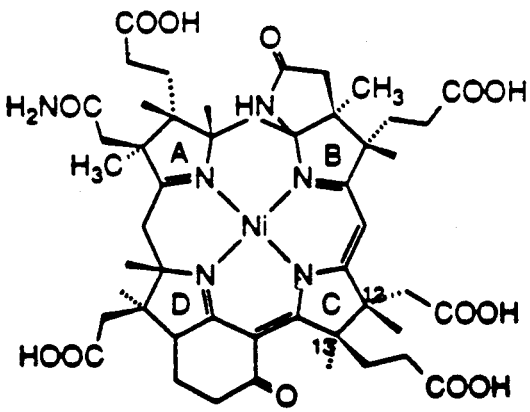
2. Calculated nickel hydrocorphinate structure.



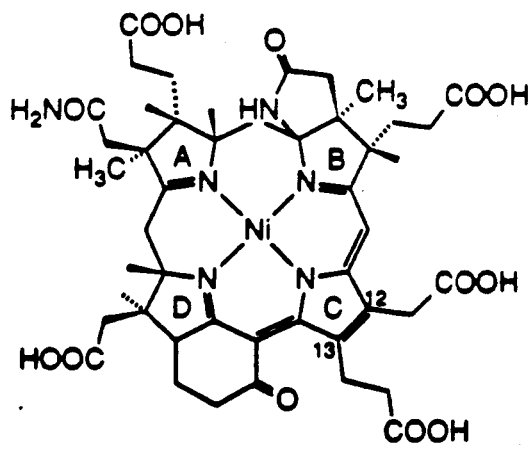
Native F_{430}



13-monoepimer

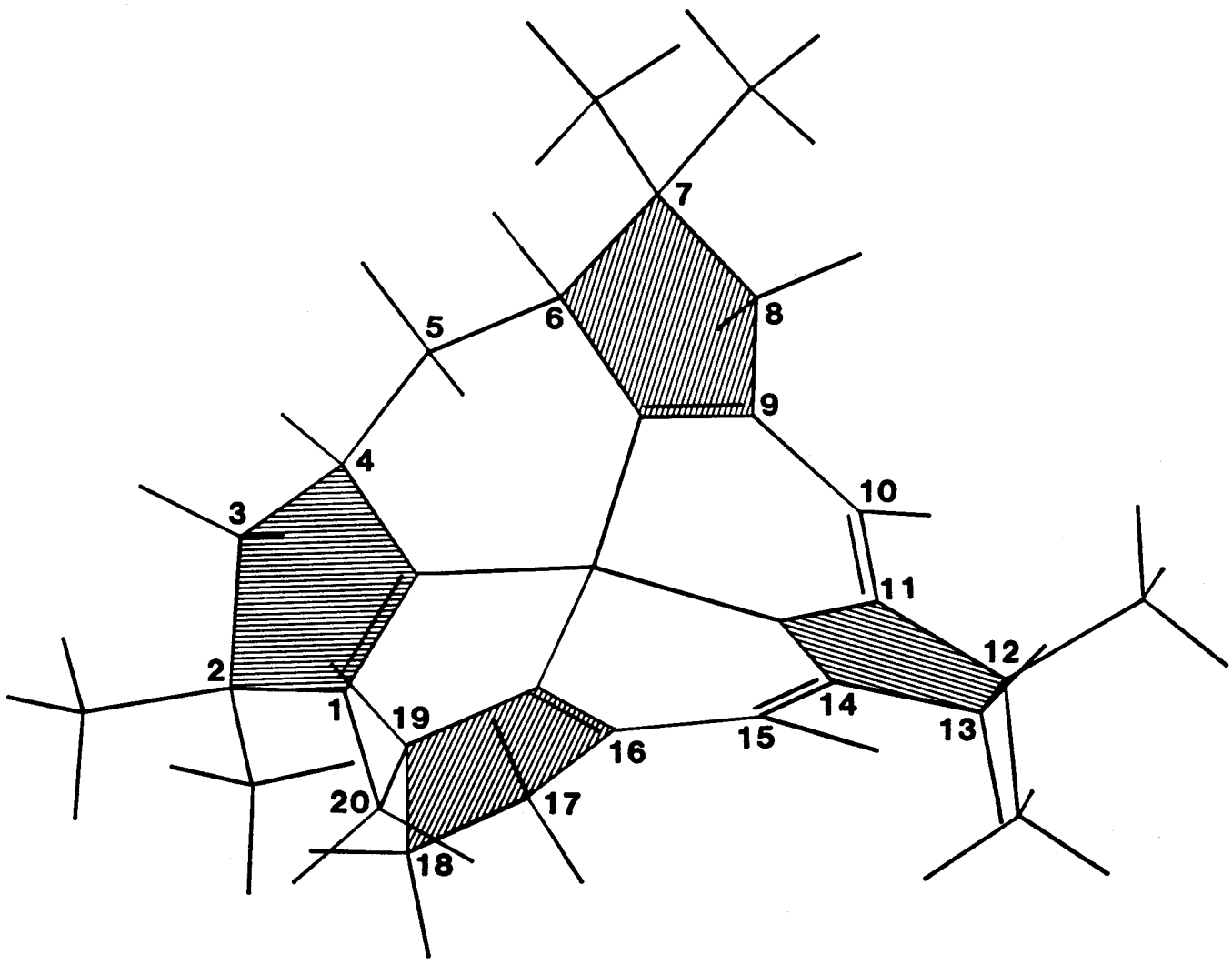


12,13-diepimer



F_{890}
(12,13-didehydro F_{430})

FIGURE 1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GRI PUBLICATIONS

1988

1. Retrievable Publications in 1988 Resulting from GRI Research

1. "Resonance Raman Spectroscopic Investigation of Axial Coordination in *M. thermoautotrophicum* Methyl Reductase and its Nickel Tetrapyrrole Cofactor F_{430}" Shiemke, A. K.; Scott, R. A.; Shelnutt, J. A., J. Am. Chem. Soc. **1988**, 110, 1645.

2. "A New Crystalline Phase of Octaethylporphinatonickel(II). Effects of π-π Interactions on Molecular Structure and Resonance Raman Spectra" Brennan, T. D.; Scheidt, W. R.; Shelnutt, J. A., J. Am. Chem. Soc. **1988**, 92, 307.

3. "Photodynamics of Ni-Porphyrins in Non-Coordinating Solvents: Characterization of d-d Excited States Using Transient Raman Spectroscopy" Findsen, E. W.; Shelnutt, J. A.; Ondrias, M. R., J. Phys. Chem. **1988**, 92, 307.

4. "Sensitization and Photoredox Reactions of Zn(II)- and Sb(V)(O)(Cl)-Uroporphyrins in Aqueous Media" Kalyanasundaram, K.; Shelnutt, J. A.; Grätzel, M., Inorg. Chem. **1988**, 27, 2820.

5. "A Resonance Raman Study of the Binding of Ethanol and Methanol to Ferrihemoglobin" Muhoberac, B. B.; Shelnutt, J. A.; Ondrias, M. R.; FEBS Letts. **1988**, 228, 310.

6. "The Photodynamics of a Nickel Hydrocorphinoid Model of F_{430}" Crawford, B. A.; Findsen, E. W.; Ondrias, M. R.; Shelnutt, J. A., Inorg. Chem. **1988**, 27, 1842.

2. Manuscripts Submitted in 1988 But Not Yet Published

7. "Multiple Four-Coordinate Forms in a Nickel Hydrocorphinate Related to Cofactor F_{430} of Methyl Reductase" Shelnutt, J. A., J. Phys. Chem. **1989**, in press.

8. "Ruffling of Nickel(II) Octaethylporphyrin in Solution" Alden, R. G.; Crawford, B. A.; Doolen, R.; Ondrias, M. R.; Shelnutt, J. A., J. Am. Chem. Soc. **1989**, in press.

9. "Structural and Spectroscopic Characterization of Exogeneous Ligand Binding to Isolated Cofactor F_{430} and its Configurational Isomers" Shiemke, A. K.; Kaplan, W. A.; Hamilton, C. L.; Shelnutt, J. A.; Scott, R. A., J. Biol. Chem. **1989**, submitted.

10. "Coordination Chemistry of Factor F_{430}: Axial Ligation Equilibrium between Square-Planar and Bis-Aquo Species in

Aqueous Solution" Shiemke, A. K.; Shelnutt, J. A.; Scott, R. A., J. Biol. Chem. **1989**, submitted.

3. Retrievable Papers Given at Symposia in 1988

1. "Ruffling of Nickel(II) Octaethylporphyrin in Solution" Alden, R. G.; Crawford, B. A.; Ondrias, M. R.; Shelnutt, J. A., Proceedings of the 11th International Conference on Raman Spectroscopy, London, Sept. 5-9, 1988.

2. "The Photodynamics of Nickel Corphin and Salt Extracted F_{430}" Crawford, B. A.; Shelnutt, J. A.; Findsen, E. W.; Ondrias, M. R., Biophys. J. **1988**, 53, 111a.

3. "An Alcohol-Induced Alteration of the Resonance Raman Spectrum of Human Ferrihemoglobin" Muhoberac, B. B.; Wells, C.; Chavez, M.; Ondrias, M. R.; Shelnutt, J. A., Biophys. J. **1988**, 53, 276a.

4. "Methyl Reductase: Comparison of Resonance Raman Spectra of the Intact Enzyme and F_{430} in Solution" Shelnutt, J. A.; Shiemke, A. K.; Scott, R. A., Biophys. J. **1988**, 53, 630a.

5. "Ruffling of Nickel(II) Octaethylporphyrin in Crystalline and Solution Phases" Alden, R. G.; Crawford, B. A.; Ondrias, M. R.; Brennan, T. D.; Scheidt, W. R.; Shelnutt, J. A., Proc.,

Natl. Am. Chem. Soc. Meeting, Los Angeles, CA, Sept. 25-30, 1988.

6. "The Role of the Solvent in the Modulation of the d-d Excited State Properties of Nickel Porphyrins in Noncoordinating Solvents as Investigated by Transient Raman and Absorption Spectroscopies" Findsen, E. W.; Shelnutt, J. A.; Ondrias, M. R., Biophys. J. **1989**, 55, 521a.

7. "Coordination Chemistry and Macrocycle Conformation of Cofactor F_{430} of Methylreductase and Related Nickel Tetrapyrroles" Shelnutt, J. A.; Shiemke, A. K.; Scott, R. A.; Crawford, B. A.; Alden, R. G.; Ondrias, M. R., Biophys. J. **1989**, 52a.

4. Papers Given at Symposia Not Yet Published

8. "Effects of π-π Dimerization and Complex Formation on Macrocycle Ruffling of Nickel Porphyrins" Alden, R. G.; Shelnutt, J. A.; Ondrias, M. R., Natl. Am. Chem. Soc. Meeting, Dallas, TX, April 9-14, 1989.

9. "Picosecond Time-Resolved Resonance Raman Study of Nickel(II) Protoporphyrin Dimethyl Ester" Alden, R. G.; Ondrias, M. R.; Courtney, S.; Shelnutt, J. A.; Friedman, J. M., Intl. Conf.

on Time Resolved Vibrational Spectroscopy, Princeton, NJ, June 11-15, 1989.

GRI PUBLICATIONS

1987

1. Retrievable Publications in 1987 Resulting from GRI Research.

1. "Heme-linked Ionizations of Myeloperoxidase Detected by Raman Difference Spectroscopy: A Comparison with Plant and Yeast Peroxidases" Stump, R. F.; Deanin, G. G.; Oliver, J. M.; Shelnutt, J. A., Biophys. J. **1987**, 51, 605-610.

2. "Axial Coordination in Nickel and Vanadium Porphyrins: Transient and Difference Raman Spectroscopy" Shelnutt, J. A.; Findsen, E. W.; Ondrias, M. R.; Alston, K., in "Metal Complexes in Fossil Fuels" eds. Filby, R. H.; Branthaver, J. (ACS Symposium Series No. 344) American Chemical Society: Washington, 1987, Chpt. 24.

3. "Nickel Site of Methane Catalysis in the Methyl Reductase Enzyme" Shelnutt, J. A.; Shiemke, A. K.; Scott, R. A., Reprints, Fuel Chem. Div., Methane Activation, ACS **1987**, 32, 272-279.

4. "Axial Ligation-Induced Structural Changes in Nickel Hydrocorphinoids Related to Coenzyme F_{430} Detected by Raman Difference Spectroscopy" Shelnutt, J. A., J. Am. Chem. Soc. **1987**, 109, 4169-4173.

3. Retrievable Papers Given at Symposia in 1987.

1. "Photodynamics of Ni-Porphyrins: Transient Raman Studies of Model Complexes and Reconstituted Hemoglobin and Myoglobin" Findsen, E. W.; Crawford, B. A.; Shelnutt, J. A.; Ondrias, M. R., Biophys. J. **1987**, 51, 290a.

2. "Axial Ligation in Nickel Porphyrins and Nickel Corphins Related to Coenzyme F_{430} of Methylreductase" Shelnutt, J. A., Biophys. J. **1987**, 51, 405a.

APPENDICES

APPENDIX 1

COORDINATION CHEMISTRY OF FACTOR F_{430}: AXIAL LIGATION EQUILIBRIUM BETWEEN SQUARE-PLANAR AND BIS-AQUO SPECIES IN AQUEOUS SOLUTION

A. K. Shiemke, J. A. Shelnutt, R. A. Scott

J. Biol. Chem. **1989**, in press.

Summary

X-ray absorption spectroscopic characterization of axial ligand coordination to factor F_{430}, the nickel-tetrapyrrole cofactor of the S-methyl coenzyme M (CH_3SCoM) methyl reductase enzyme from methanogenic bacteria, is presented. The nickel of isolated F_{430} is hexacoordinate at 10K in aqueous solution (as is the enzyme-bound cofactor), whereas the epimerized and ring-oxidized derivatives of F_{430} have 4-coordinate nickel. Reduction of the ring-oxidized derivative, F_{560}, with dithionite yields F_{430} in its native configuration, with axial ligands identical to those present when the cofactor is obtained directly from the holoenzyme. Thus, we conclude that the axial ligands to F_{430} in aqueous solution are water molecules. Analysis of the Ni extended x-ray absorption fine structure (EXAFS) is consistent with this conclusion. Resonance Raman spectra obtained at room temperature contain features characteristic of both four- and six-coordinate forms of the cofactor. We have found that the resonance Raman, optical, and x-ray absorption spectra of aqueous solutions of F_{430} are temperature dependent due to a ligand-binding equilibrium involving the square-planar and six-coordinate bis-aquo forms of the cofactor. At low temperatures (< 250 K) the six-coordinate form predominates, whereas higher temperature solutions contain both four- and six-coordinate species in a dynamic equilibrium. Similar behavior is observed in other weakly coordinating solvents such as methanol and ethanol. The four-coordinate form is predominant in solvents with strong electron withdrawing substituents such as 2,2,2-trifluoroethanol and 2-mercaptoethanol. The relevance of this facile ligand exchange to the active site structure and enzymatic mechanism of the parent enzyme is discussed.

APPENDIX 2

STRUCTURAL AND SPECTROSCOPIC CHARACTERIZATION OF EXOGENOUS LIGAND BINDING TO ISOLATED COFACTOR F_{430} AND ITS CONFIGURATIONAL ISOMERS

A. K. Shiemke, W. A. Kaplan, C. L. Hamilton,

J. A. Shelnutt, R. A. Scott

J. Biol. Chem. **1989**, in press.

Summary

Binding of axial ligands to the nickel of isolated cofactor F_{430} from the methyl reductase enzyme of *Methanobacterium thermoautotrophic ..* is demonstrated. Evidence of bis-ligand coordination is obtained from the x-ray absorption, optical, and resonance Raman spectral characterization of F_{430}, and its 12,13-diepimeric isomer, in the presence of a large excess of cyanide, pyridine or 1-methylimidazole. Significant broadening and 5-10 nm red shifts of the main 430 nm optical absorption band are observed upon coordination of these axial ligands. Shifts of up to 30 cm^{-1} are observed for high frequency Raman lines upon conversion of the nickel geometry from square-planar in the diepimer to octahedral in the bis-ligand complex. The Raman spectra of native F_{430} and the diepimer with a particular axial ligand are nearly identical. X-ray absorption edge spectra of the diepimer in the absence and presence of these exogenous ligands are also indicative of a square-planar to pseudo-octahedral conversion. Analyses of the EXAFS data for the ligated diepimer complexes yield detailed structural information for these complexes. Implications of these data with respect to the enzymatic mechanism and the structure of the enzyme-bound cofactor are discussed.

APPENDIX 3

MULTIPLE FOUR-COORDINATE FORMS IN A NICKEL HYDROCORPHINATE RELATED TO COFACTOR F_{430} OF METHYLREDUCTASE

J. A. Shelnutt

J. Phys. Chem. **1989**, in press.

ABSTRACT

Four and six coordinate forms of a nickel(II)-hydrocorphinate model of cofactor F_{430} of the methyl-coenzyme M methylreductase were investigated using resonance Raman spectroscopy. The six-coordinate complexes, formed in various coordinating solvents and in non-coordinating solvents with added organic bases, have Raman spectra showing only a single species at room temperature and at 77° K in frozen solutions. The six-coordinate complexes are characterized by the two highest frequency, strong lines in the 1550-1560 and 1620-1630 cm^{-1} regions. There is some variation in the frequencies of these two marker lines with the particular axial ligand, but the separation of the lines remains relatively constant at 67-74 cm^{-1}. The separation of these two lines is an indicator of the nickel coordination number for the hydrocorphinate. The separation for a five-coordinate complex is 81 cm^{-1}, and for the major four-coordinate form the separation is about 93 cm^{-1}. Raman spectra of four-coordinate nickel hydrocorphinates show evidence of multiple species both at room temperature and at 77° K. Besides the previously identified four-coordinate form, we have now identified two other forms. One of the newly identified species is detected in room temperature Raman spectra taken with pre-resonance excitation and is observed also in frozen solutions. The other new four-coordinate form is positively detected only in frozen samples. The new room-temperature form has a red shifted absorption spectrum based on resonance enhancement of its Raman scattering with excitation to the red of the absorption maximum. This red shift is consistent with the observed 20-nm red shift in the absorbance maximum upon freezing at 77°

K, under which conditions the new species dominate. A structural interpretation of the four-coordinate forms is proposed based on molecular mechanics calculations for the nickel-hydrocorphinate enantiomers. Ruffling of the macrocycle is proposed to account for the new conformers.

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